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Bioactivity of $CaO \cdot SiO_2$ glasses added with various ions

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The purpose of this investigation was to study the fundamental compositional dependence of bioactivity for bioactive glasses by adding a third component such as Na_2O, B_2O_3, Al_2O_3, Fe_2O_3, P_2O_5 or F^- to binary calcium silicate glass. Rectangular specimens of these six kinds of calcium silicate glasses containing each 3 wt ratio third component were implanted into the tibias of mature rabbits for 8 or 25 weeks. Contact microradiograms and SEM-EPMA showed that all glasses, except for Fe_2O_3-containing glass, formed a Ca, P-rich layer in combination with a Si-rich layer on their surfaces within 8 weeks. The other four kinds of glass except that containing Al_2O_3 became attached to bone through these layers within 8 weeks, and even Al_2O_3-containing glass became attached to bone by 25 weeks. Fe_2O_3-containing glass did not form these layers, nor did it attach to bone even at 25 weeks. A detachment test, performed 8 weeks after implantation, showed that only glasses containing B_2O_3, P_2O_5 and F^- bonded tightly with bone. Glass containing B_2O_3 maintained a tight bond at 25 weeks, but the other two kinds of glass showed decreased loads at 25 weeks.

1. Introduction

Since the development of Bioglass, many bioactive glasses and glass-ceramics in the system CaO–SiO_2–P_2O_5 have been produced [1–4]. The compositional dependence of bone–Bioglass bonding has been investigated in detail. However, the extent to which each component of Bioglass influences the bone bonding phenomenon has not been clarified [5–10]. Since Bioglass consists of at least four components (CaO, SiO_2, Na_2O and P_2O_5), it is difficult to study their mutual action precisely.

Recently, we reported that a binary calcium silicate glass (CS glass, CaO 50(48.3), SiO_2 50(51.7) in mol % (in wt %)) also formed a Si-rich layer and a Ca, P-rich layer on its surface, and bonded with bone tissue [11]. This binary glass is one of the simplest bioactive silicate glasses. Therefore, it is considered that, by adding a third component to this glass, its influence on the bone bonding of bioactive glasses can be examined.

The purpose of the present study was to examine how much influence would be exerted on the bonding between bone and binary calcium silicate glass when 3 wt ratio of each of Na_2O, B_2O_3, Al_2O_3, Fe_2O_3, P_2O_5 and F^- was added to calcium silicate glass.

2. Materials

Six kinds of glasses were prepared: (1) CS–Na glass, (2) CS–B glass, (3) CS–Al glass, (4) CS–Fe glass, (5) CS–P glass, (6) CS–F glass.

The compositions of these glasses are listed in Table I. Glass batches were prepared from reagent-grade $CaCO_3$, SiO_2, Na_2CO_3, $H_3B_2O_3$, Al_2O_3, Fe_2O_3, $CaHPO_4\ 2H_2O$ and CaF_2. Premixed batches were melted in a platinum crucible at 1550 °C for 1 h, poured onto steel plates and pressed into glass plates. The glasses were cut into $15 \times 10 \times 1\ mm^3$ plates and polished with 1 μm diamond paste on their surfaces.

3. Method

The rectangular glass plates were cleaned in an acetone-filled ultrasonic cleaner for 20 min, and conventionally sterilized with a drying sterilizer at 180 °C for 1 h. The plates were then implanted into the metaphyses of the tibias of male rabbits weighing between 3.0 and 3.5 kg. The operative method was the same as that reported previously [12]. The animal experiments were carried out at the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University, CS–Na, CS–B, CS–Al, CS–Fe, CS–P and CS–F glasses were each implanted into six animals.

Three animals from each group were slaughtered eight weeks after implantation and the remaining three were slaughtered after 25 weeks. A segment of each tibia containing the implant was excised, and for

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TABLE I Chemical composition of six kinds of glasses

Name	Composition (in wt ratio)
CS–Na	CaO 48.3, SiO_2 51.2, Na_2O 3.0
CS–B	CaO 48.3, SiO_2 51.2, B_2O_3 3.0
CS–Al	CaO 48.3, SiO_2 51.2, Al_2O_3 3.0
CS–Fe	CaO 48.3, SiO_2 51.2, Fe_2O_3 3.0
CS–P	CaO 48.3, SiO_2 51.2, P_2O_5 3.0
CS–F	CaO 48.3, SiO_2 51.2, CaF_2 6.0 (F^- 2.9)

each specimen the failure load necessary for detaching the implant from the bone was measured using Nakamura's method [12].

After the detachment test, each specimen was fixed with 10% phosphate-buffered formalin solution. After dehydration with ethanol, the specimens were embedded in polyester resin. Thin sections were cut with an EXAKT-Cutting-Grinding System and ground to about 150 μm thickness for contact microradiography and Giemsa surface staining Speed Lap (Maruto Ltd, Tokyo, Japan). The surfaces for SEM-EPMA (scanning electron microscope and electron probe microanalysis) were made by polishing sections 5 mm thick with diamond paste. Before analysis, these samples were coated with a thin layer of carbon (– 100 Å). They were then analysed for the X-ray intensities of calcium, silicon and phosphorus at the implant-bone interface by using a scanning electron microscope (Shimadzu Seisakujo Ltd, ASM-SX, Kyoto, Japan) connected to an energy dispersive system (EG&G ORTEC, TN, USA). An electron beam, 0.01 μm in diameter, maintained at 3×10^{-10} A, was used and the X-ray intensities in counts per second (CPS) were recorded. The accelerating voltage was 15 kV [13].

4. Results

4.1. Biocompatibility of glasses

Contact microradiograms showed the interface between the bone and each type of glass (Figs 1–6). After eight weeks, new bone reached the surface of each glass and spread over their surface. Except CS–Al and CS–Fe glasses, the surfaces of other four kind glasses became irregular and were covered with broad high-density film. However, the surfaces of CS–Al and CS–Fe glasses were still smooth and there existed a gap between these glasses and bone.

After 25 weeks, the appearance varied considerably among the glasses. In the case of CS–B, CS–P and CS–F glasses, the distances between glass surfaces and bone were unchanged. However, bone invaded into CS–P glass and bone trabeculas had disappeared over CS–F glass surface. Only CS–B glass showed the same appearance as that at eight weeks. The low-density zone between bone and CS–Na glass widened at 25 weeks. The gap between CS–Al glass and bone became narrower at a few points, and the glass surface became rougher than that at eight weeks. There still existed a gap between CS–Fe glass and bone.

SEM-EPMA showed the bone–glass interface (Figs 7–12). Except CE–Fe glass, each glass formed a Si-rich layer on its surface and a Ca, P-rich layer between the Si-rich layer and the bone within eight weeks. Glasses CS–Na, CS–B, CS–P and CS–F became attached directly to the bone through these layers by eight weeks, but CS–Al glass took 25 weeks. Only CS–Fe glass failed to form these layers, and had not attached to the bone by 25 weeks. The widths of both the Si-rich

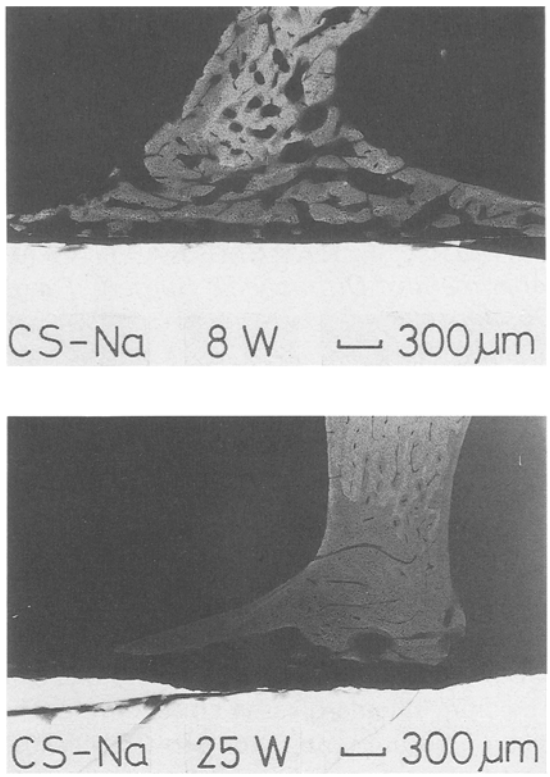

Figure 1 Contact microradiograms of CS–Na glass.

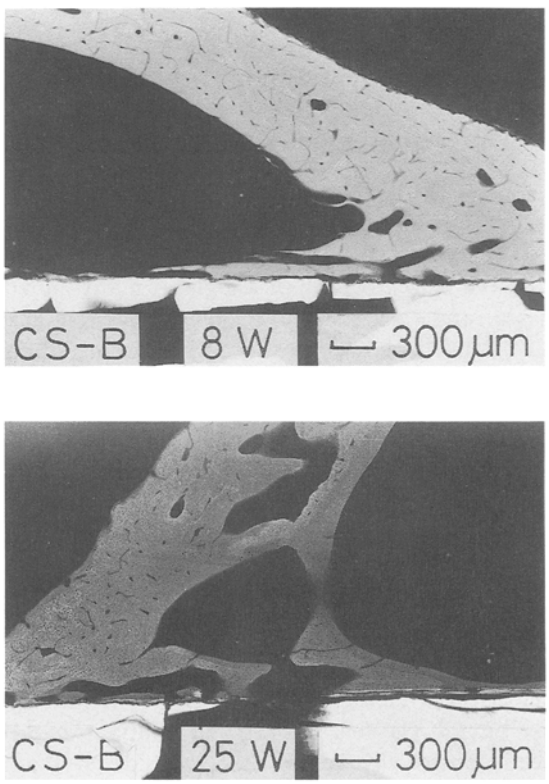

Figure 2 Contact microradiograms of CS–B glass.

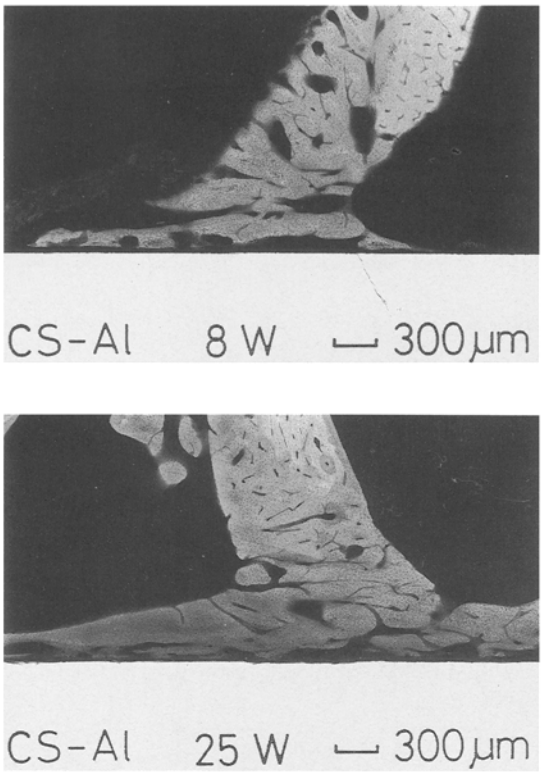

Figure 3 Contact microradiograms of CS–Al glass.

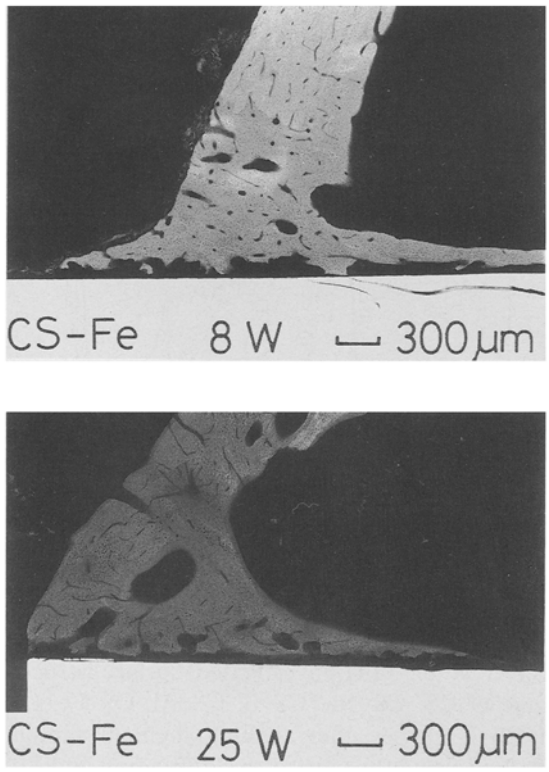

Figure 5 Contact microradiograms of CS–P glass.

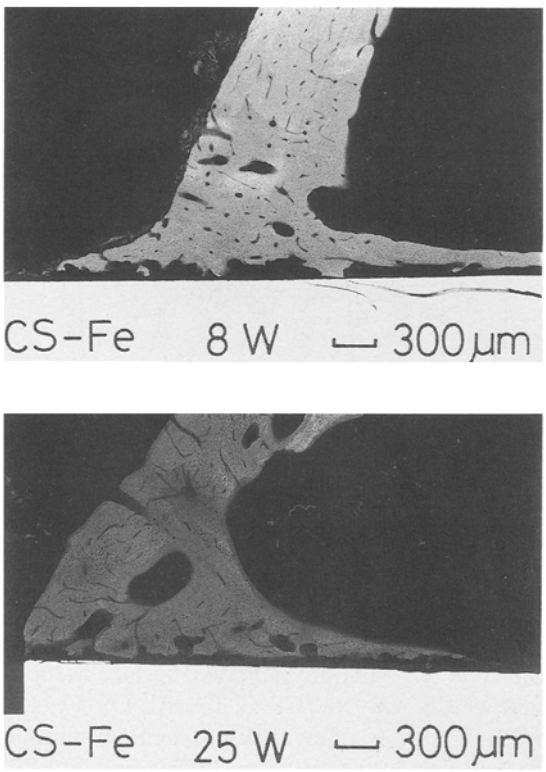

Figure 4 Contact microradiograms of CS–Fe glass.

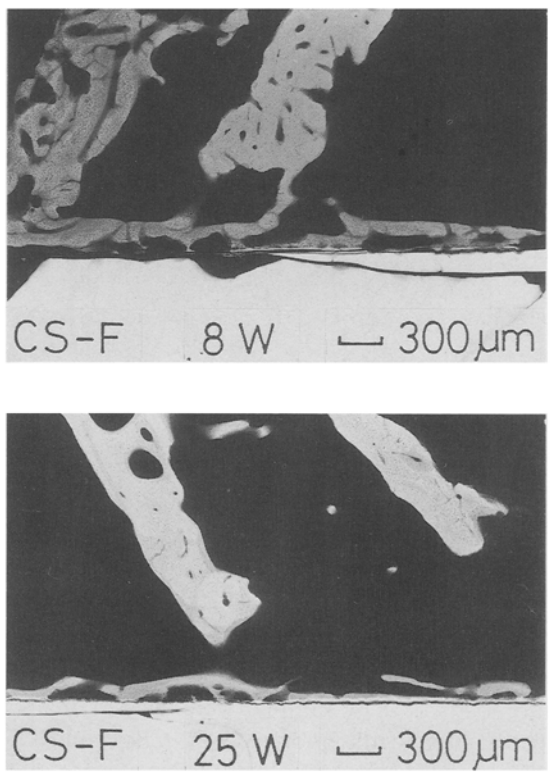

Figure 6 Contact microradiograms of CS–F glass.

layer and the Ca, P-rich layer (reaction zone) of CS–Na, CS–B, CS–Al, CS–P and CS–F glasses were 23–31 μm, 20–34 μm, 20–28 μm, 23–31 μm and 24–31 μm at eight weeks, 70–90 μm, 15–20 μm, 10–14 μm, 18–30 μm and 22–34 μm at 25 weeks, respectively.

4.2. Evaluation of bone bonding of glasses

Table II summarizes the failure loads of each glass. All of CS–Al and CS–Fe glasses, one CS–Na glass at eight weeks, and two CS–Na and three CS–F glasses at 25 weeks were detached from bone tissues before the

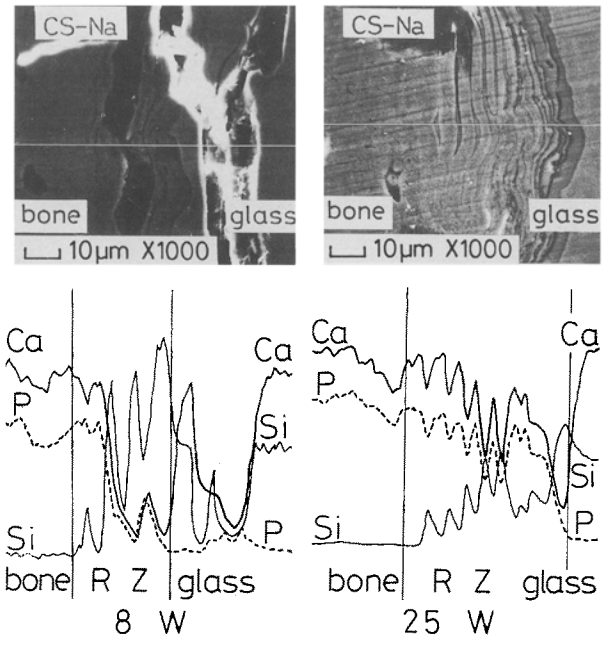

Figure 7 SEM-EPMA of CS–Na glass (Ca, P: 1000 CPS, Si: 3000 CPS).

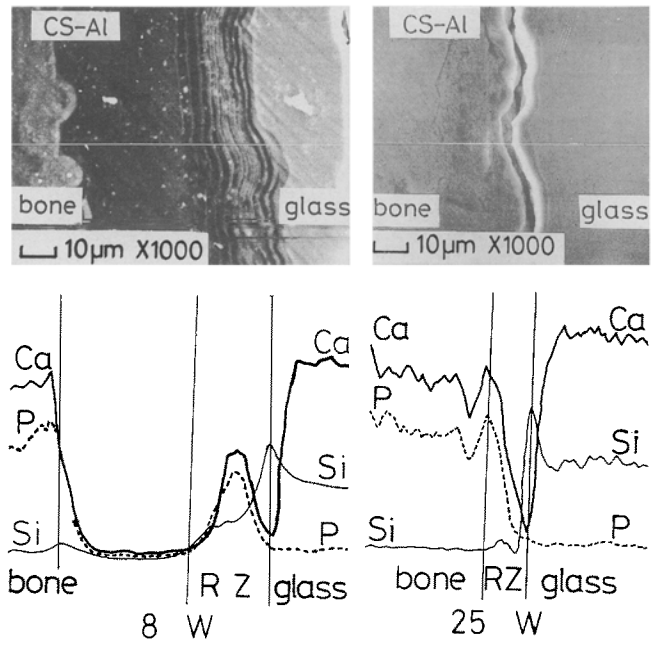

Figure 9 SEM-EPMA of CS–Al glass (Ca, P: 1000 CPS, Si: 3000 CPS).

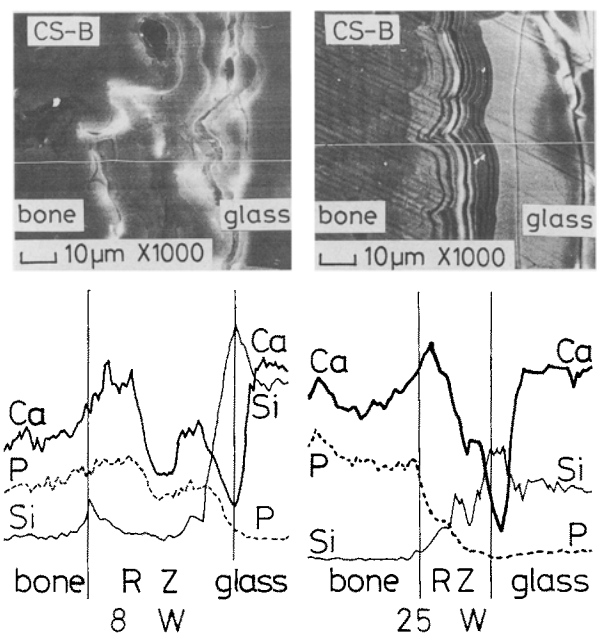

Figure 8 SEM-EPMA of CS–B glass (Ca, P: 1000 CPS, Si: 3000 CPS [8W], 3000 CPS [25W].

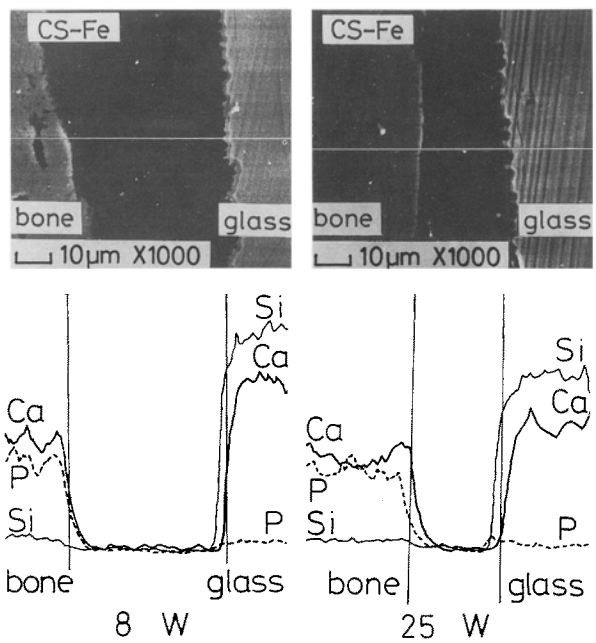

Figure 10 SEM-EPMA of CS–Fe glass (Ca, P, Si: 1000 CPS).

detachment test. Furthermore, one CS–P glass specimen dissolved completely by 25 weeks. Therefore, the failure loads of these samples were defined as 0 kg.

The failure loads among CS–B, CS–P and CS–F glasses, eight weeks after implantation, were almost the same, and higher than those of CS–Na, CS–Al and CS–Fe glasses. The loads for CS–P and CS–F glasses decreased with time, but those for CS–B glass did not decrease.

Giemsa surface staining of CS–F glass 25 weeks after implantation showed that small bone fragments were attached to the glass, but that bone trabeculas over the glass had disappeared and changed into fibrous tissue (Fig. 13).

5. Discussion

Ebisawa *et al.* [14] investigated surface structural change of CS, CS–Na, CS–B, CS–Al, CS–Fe, CS–P and CS–F glasses after soaking them in simulated body fluid using thin-film X-ray diffraction and Fourier transform infrared reflection spectroscopy. Glasses CS, CS–Na, CS–P, CS–B, CS–F and CS–Al were covered with a layer of apatite in 1, 1, 1, 7, 20, 30 days, respectively. CS–Fe glass did not form these layer by 30 days [14, 15]. These results show that CS glasses become more reactive following addition of Na_2O and P_2O_5, but less reactive following addition of B_2O_3, F^-, Al_2O_3 and Fe_2O_3. Taking these results into consideration, we investigated the reactivity of six kinds of

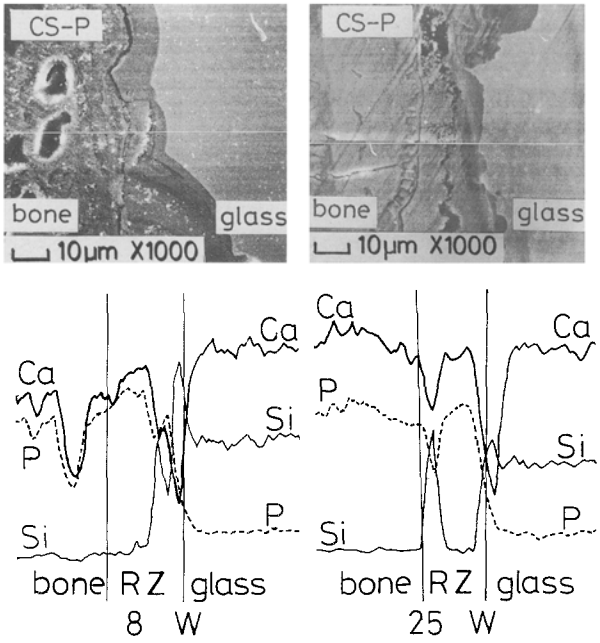

Figure 11 SEM-EPMA of CS–P glass (Ca, P:1000 CPS, Si: 3000 CPS).

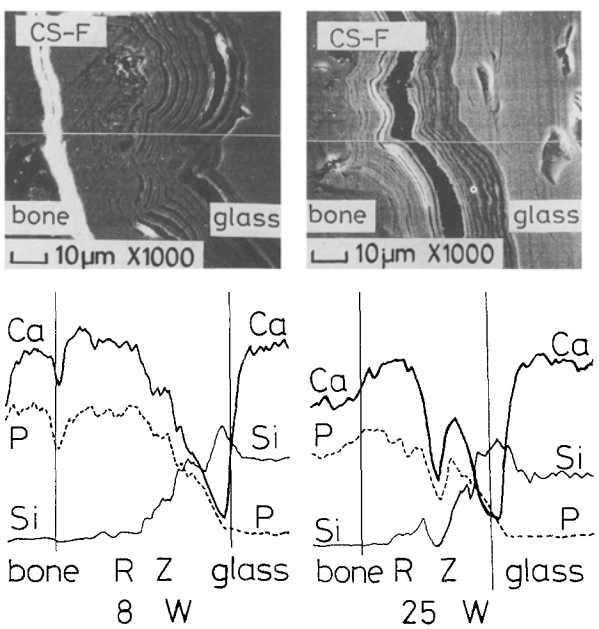

Figure 12 SEM-EPMA of CS–F glass (Ca, P:1000 CPS, Si: 3000 CPS).

CS glasses *in vivo*. SEM-EPMA showed that CS–Na, CS–B, CS–Al, CS–P and CS–F glasses formed a Si-rich layer and a Ca, P-rich layer in eight weeks. However, only CS–B, CS–P and CS–F glasses bonded tightly with bone at eight weeks. Glass CS–Na attached to the bone through these layers in eight weeks, but was very weak at bone bonding. Increasing the Na_2O content at the expense of CaO to calcium silicate glasses it is thought that a more collosive glass will result [16]. So only CS–Na glass formed a wide Si-rich layer like Bioglass, which weaken the failure load [12]. Among five CS glasses which formed the reaction zone, CS–Na glass widened the Si-rich layer in the long term, but other four kind of glasses did not change the width of this zone. The addition of Na_2O

TABLE II Failure loads measured by detachment test 8 and 25 weeks after implantation (mean + SD (kg)

Name	8 Weeks (no. of samples)	25 Weeks (no. of samples)
CS–Na	0.16 + 0.08 (6)	0.13 + 0.08 (6)
CS–B	1.01 + 1.01 (6)	1.18 + 1.47 (6)
CS–Al	0 (6)	0 (6)
CS–Fe	0 (6)	0 (6)
CS–P	1.80 + 1.13 (6)	0.43 + 0.46 (6)
CS–F	1.49 + 0.98 (6)	0.02 + 0.03 (6)

Student's *t*-test.
CS–B versus CS–P versus CS–F at 8 weeks: not significant at $p < 0.01$.
CS–B, CS–P, CS–F versus CS–Na at 8 weeks: significant at $p < 0.05$.
CS–B 8 weeks versus 25 weeks: significant at $p < 0.05$.
CS–P 8 weeks versus 25 weeks: not significant at $p < 0.05$.
CS–F 8 weeks versus 25 weeks: significant at $p < 0.05$.

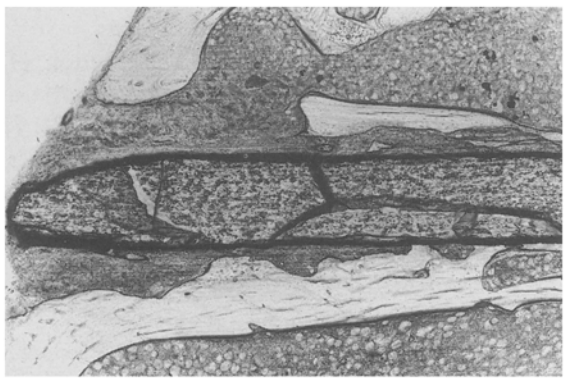

Figure 13 Giemsa surface staining of CS–F glass 25 weeks after implantation.

to CS glass is supposed to be the main factor to widen the Si-rich layer of CS glasses. Glass CS–B maintained the large failure loads at 25 weeks, but CS–P and CS–F glasses showed decreased failure loads at 25 weeks. CS–P glass continued to dissolve after bonding to the bone. The glass specimen became small and its bone-bonding area decreased 25 weeks after implantation. Thus the failure loads of this glass decreased. Addition of only 3 wt ratio of P_2O_5 to CS glass increased not only the reactivity but also the solubility of calcium silicate glass to an excessive degree. CS–F glass was encapsulated by bone within eight weeks, but the bone tissue over the glass disappeared and changed into fibrous tissue at 25 weeks. Thus the failure loads of this glass decreased. Fluoride increases bone formation at low concentration, but has a toxic effect on bone tissue at high concentration [17]. It is supposed that the containment of as much as 6 wt ratio of CaF_2 is too much to allow CS glass to maintain good bone formation in the long term. Only CS–B glass was given appropriate reactivity and solubility by addition of 3 wt ratio B_2O_3, so that this glass bonded fast with the bone and maintained a large failure load for a long period.

CS–Al glass formed the Si-rich and Ca, P-rich layers on its surface, but did not attach to bone within eight weeks. By 25 weeks, this glass had directly attached to

bone through these layers. However, the bone attachment area was so small that the bone was detached from all glass surfaces before the detachment test. It has been reported that addition of Al_2O_3 destroys the bioactivity by an increased corrosion resistance and precipitation of multivalent ions as oxides, hydroidea or carbonates at pH values below those required for calcium phosphate precipitation [18, 19]. The addition of 3 wt ratio of Al_2O_3 to CS glass was not enough to prevent formation of the Ca, P-rich layer, but sufficient to decrease the volume of this layer on CS glasses.

CS–Fe glass did not form a Si-rich layer nor a Ca, P-rich layer even at 25 weeks after implantation. Fe_2O_3 is known as a component effective for improving chemical durability [20]. These results indicate that addition of Fe_2O_3 to CS glass suppresses formation of these layers more strongly than that of Al_2O_3, and addition of even 3 wt ratio Fe_2O_3 was enough to completely prevent layer formation.

Kokubo *et al.* reported that the formation of a surface apatite layer was thought to be essential for direct bonding of bioactive ceramics to bone [21, 22]. In this study, we have revealed that all five kinds of CS glasses which formed the Ca, P-rich layer on its surface, bond with bone. Therefore, it can be speculated that we have confirmed their hypothesis *in vivo*.

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